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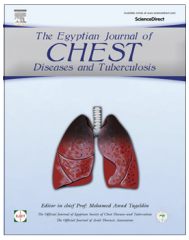

ORIGINAL ARTICLE

Assessment of diagnostic accuracy of Gene Xpert MTB/RIF in diagnosis of suspected retreatment pulmonary tuberculosis patients

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KEYWORDS

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Abstract *Background:* Retreatment cases of tuberculosis are reemerging as public and global health concerns, not only for dissemination of infection but for their multidrug resistant behavior as well, comprising an important challenge for National Tuberculosis Control Programs. Gene Xpert MTB/RIF is an automated molecular test for synchronized detection of tuberculosis and rifampicin resistance, recommended by the World Health Organization. This study was designed to assess the performance of single-sputum Gene Xpert MTB/RIF assay against the Ziehl–Neelsen smear, traditional culture and MGIT 960 in diagnosing suspected retreatment pulmonary tuberculosis cases and rifampicin resistance.

Subjects and methods: Fifty-eight patients were enrolled in this cross-sectional study from two Egyptian tertiary care hospitals. The patients were selected fulfilling clinical criteria of suspected retreatment TB. Early-morning sputum specimens were collected, decontamination liquefaction technique was done, the specimen was divided into four portions, the first and second portions were for the Ziehl–Neelsen smear and cultivation on the Löwenstein Jensen media. The third portion was processed in Gene Xpert MTB/RIF. The fourth part was used for confirmation of rifampicin susceptibility testing results by MGIT 960.

Results: Gene Xpert MTB/RIF showed sensitivity, specificity and accuracy of 98.15%, 75% and 96.55% respectively in detection of mycobacterium tuberculosis. Regarding rifampicin mono-resistance, it carried the best sensitivity and specificity of 100% equal to that of reference test MGIT 960.

Conclusion: Gene Xpert MTB/RIF is a sensitive, specific and accurate test for both diagnosis of retreatment MTB and rifampicin resistance. Finally the authors recommend restandardization of reference test with Gene Xpert MTB/RIF to obtain more valid results.

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Introduction

As stated by WHO, Egypt is ranked with middle/low level of tuberculosis (TB) incidence countries, however no available figure about retreatment TB cases. A multinational study was carried out under WHO supervision in 2012 comprising high burden countries, about 6.1 million with TB were notified to National TB Program. Of these, 0.3 million had a recurrent episode of TB after treatment and 0.4 million had already been diagnosed with TB and engaged into a retreatment regimen [1]. Recurrence of active TB after treatment happens either due to relapse of infection with the same strain or reinfection with a new strain of mycobacterium TB (MTB). The proportion of recurrent tuberculosis cases caused by reinfection has varied widely in previous studies [2]. Yet, retreatment outcomes are often poor, especially in patients with treatment failure or default [3]. An alarming rise in the global incidence of infections caused by MTB has prompted the need for rapid diagnostic techniques [4]. Conventional diagnostic methods for MTB are slow and/or lack sensitivity [5]. Gene Xpert MTB/RIF assay is an automated, closed-cartridge system, easy to operate and user friendly. It is based on a hemi nested real-time PCR assay utilizing five molecular beacon technology spanning the rpoB gene 81-bp rifampicin resistance-determining region (RRDR), the test concurrently determines MTB and rifampin susceptibility, which can be used as a surrogate marker for multidrug resistance (MDR–TB) [6]. The results are obtained within a short period of 2 h [4]. This study was designed to assess the performance of single-sputum Gene Xpert MTB/RIF assay against the Ziehl–Neelsen smear, traditional culture and MGIT 960 in diagnosing suspected retreatment pulmonary tuberculosis cases and rifampicin resistance (see Tables 1–4).

Table 1 Demographic characteristics of all studied patients.

Parameter	No	%
Age (years) (Mean ± SD)	32.52 ± 19	
Sex		
Male	39	67.2
Female	19	32.75
Smoking history		
Ex- smoker	14	24.1
Current smoker	29	50
Presence of comorbidity	23	39.6
Use of corticosteroids	34	58.6

Table 2 Comparison between different TB diagnostic methods.

	ZN smear +ve	LJ media culture +ve	Gene Xpert +ve
Sputum sample	49	54	54

Study design and setting

A cross sectional study was carried out over one year duration from November 2014 to October 2015 in both Chest Department and Medical Microbiological and Immunology Department, Zagazig University and General Chest Hospital. The University Ethics Committee approved this study. Full written consent was taken from all patients enrolled in this study before history taking or specimen collection.

Patients and methods

This prospective study included 58 suspected (needed to be confirmed as retreatment cases bacteriologically) retreatment tuberculous outpatients. They asked medical advice at outpatient clinic of Zagazig University Hospitals and Chest Hospital, Zagazig, Sharkia Governorate, Egypt, for initial clinical, radiological and microbiological assessment and treatment. The diagnosis of retreatment pulmonary TB was, as stated by National TB Control Program of Egypt, [7] on the basis of:

1. History of receiving antituberculous drugs for more than one month.
2. Recurrence of general and/or local chest symptoms.
3. Documented positive Ziehl–Neelsen (ZN) smear or culture on the Lowenstein–Jensen (LJ) media for acid- fast bacilli (AFB) after appearance of symptoms.

Case definition

These retreatment cases include patients with treatment failure, relapse and defaulters according to Instructions for completion of the WHO/Euro TB data collection form for TB cases, 2007 [7].

Treatment failure: is defined as a patient previously treated for TB but who remained sputum smear positive at five months or later during treatment.

Relapse: is defined as a patient previously treated for TB and declared cured or who completed treatment, and was diagnosed with sputum smear positive TB.

Defaulter: is defined as a patient previously treated for TB but who had interrupted treatment for two or more consecutive months.

Table 3 Results of culture on LJ medium and Gene Xpert MTB/RIF regarding to ZN smear.

Smear result	Results of LJ culture and Gene Xpert		
	LJ culture +ve and Gene Xpert +ve	LJ culture −ve and Gene Xpert +ve	LJ culture −ve and Gene Xpert −ve
ZN smear +ve	49	0	0
ZN smear −ve	5	1	3

Table 4 Sensitivity and specificity of Gene Xpert MTB/RIF results in comparison to LJ culture results.

Parameter	LJ culture (54)		Total	Sensitivity 95% Confidence interval	Specificity 95% Confidence interval	PPV	NPV	Accuracy
	+ ve	− ve						
GeneXpert	53	1	58	98.15% 0.88816–0.999	75% 0.219–0.986	98.15%	75%	96.55%

All studied patients were subjected to:

(1) Thorough medical history stressing on general and local chest symptoms, history of diabetes mellitus (DM), receiving corticosteroid therapy, associated autoimmune disorders and history of antituberculous drugs in the previous course.
(2) Full clinical examination (general and local).
(3) Chest X-ray (Posteroanterior and lateral views) to be compared with the previous chest X-ray to detect new radiological data.
(4) Blood chemistry:
   – Complete blood count (CBC).
   – Erythrocyte sedimentation rate (ESR).
   – Liver and kidney functions.

(5) Microbiology specimen processing:

Early morning spontaneously produced sputum specimens were taken from all of the studied patients for 3 successive days, if it was difficult, sputum induction by inhalation of nebulized 5–10% hypertonic saline for 20 min was done [8].

Specimen collection: Early morning sputum was collected bedside in a sterile screw-capped container with rapid delivery to the laboratory for fast processing.

All the sputum specimens were subdivided into four portions to be subjected to the following:

I. *ZN staining for smear microscopy*, smears were repeated on the next two days.
II. *Culture on Löwenstein Jensen (LJ) media:* after liquefaction decontamination technique, sputum samples were cultured on LJ media slopes under 37 °C. Culture was not considered negative except after 8 weeks. Sputum culture was considered the gold standard technique in this work.
III. *Gene Xpert MTB/RIF PCR test:* (Cepheid, Sunnyvale, CA, USA): according to manufacture instruction, sputum specimens were vortexed for 1 min with glass beads and split, aliquots were frozen at −70 °C to be finally analyzed by the Xpert MTB/RIF assay, according to manufacturer's instructions. Sample reagent was added in a 2:1 ratio to sputum in 15 ml falcon tube and the tube was manually agitated twice during a 15 min-incubation period at room temperature. Then 2 ml of the inactivated material was transferred to the test cartridge by a sterile disposable pipette (provided with kits). Cartridges were loaded into the Gene Xpert. At the end of the real-time PCR run, the Xpert MTB/RIF assay's data analysis algorithm identified a specimen as MTB positive if at least two of the five rpoB probes were positive within two cycles of each other. Failure of one or more of the rpoB-specific molecular beacons to hybridize to the rpoB amplicon was interpreted as rifampin resistance. Data interpretation was received after completion of the PCR run through computed software.
IV. Detection of rifampicin resistance: by the reference automated broth-based MGIT 960, the high-level rifampicin-resistant strains (resistant to 250 μg/ml) and low-level rifampicin-resistant strains (resistant to 50 μg/ml) were detected. A 500 μl sample was inoculated in MGIT 960. After the culture flashed positive, streptomycin, isoniazid, rifampin and ethambutol (SIRE) MGIT-DST were performed along with the manufacturer's protocol.

Statistical analysis

Patient data, ZN smear results, culture findings, susceptibility results of MGIT 960 and Xpert MTB/RIF PCR data were collected, tabulated and analyzed using SPSS version 19 statistical package. In the statistical analysis, frequencies, mean values and percentages were presented. Odds ratio and chi-square were used to compare variables. Sensitivity, specificity and accuracy were calculated according to equations.

Results

Out of 58 patients (with age range 21–67 years) clinically suspected to have pulmonary retreatment TB, Z–N smear examination was positive for AFB in 49 (90.7%). L–J Culture results revealed positive yield in 54 cases. In addition to positive results for MTB, detection by Gene Xpert MTB/RIF was in 54 of cases. The present study clarified that Gene Xpert detected an extra positive retreatment tuberculosis case as compared with culture on LJ media as the gold standard test. It can detect more five positive TB cases when compared with ZN microscopy indicating a higher sensitivity of 98.15%, specificity was 75%. Another negative case was detected in comparison with LJ culture. Thirty-seven isolates (68.5%) were resistant to rifampicin by both MGIT 960 and Gene Xpert MTB/RIF.

Discussion

After the WHO's endorsement of Gene Xpert assay in 2010, different research studies have been conducted to examine Gene Xpert's utility in different countries and populations. Yet up to our knowledge, no sufficient data are available regarding retreatment tuberculosis cases. As literature studies were covering active cases of TB, MDR–TB, extrapulmonary

TB (EPTB) or associated TB in HIV cases, nonetheless, this study adheres sticky to retreatment cases as a challenging resistant population to antituberculous treatment in Egyptian communities.

Detection of retreatment cases of tuberculosis is essential for National Control Program, nevertheless it is often confronted by the limitation of transport and specimen storage from other locations to the reference laboratory. Coupled with underestimated case reporting owing to patient incompliance either to history giving or regular visits for drug intake.

In the current study, sharp differentiation of patients into clinical types could not be met, as a consequence of misleading data offered by some patients. Diagnosis of retreatment cases rested on comprehensiveness of clinicians, available medical records and interpretation of patient history to attain meaningful conclusions.

Xpert MTB/RIF assay is a double-edged weapon, indicates the presence or absence of M. tuberculosis, in a semi quantitative approximation of concentration and the presence or absence of RIF resistance [9].

This work revealed that all smear positive specimens showed positive yield on culture, and Gene Xpert MTB/RIF assay's results were positive (100%). It is evidenced that Gene Xpert MTB/RIF can detect MTB in five out of the remaining nine smear negative specimens.

ZN smear microscopy despite of being the available screening tool, it carries the risk of false negative results and incompetency to discriminate between drug susceptible and drug resistant strains of MTB. These are owing to poor sample quality coupled with a need for an experienced specialist. Meanwhile, culture being the gold standard for detecting MTB, proceeds for weeks up to months to yield results, and depends on sophisticated laboratory facilities and skilled technicians [10,11].

The out performance of Xpert MTB/RIF detected in current work is in agreement with other researchers who established the diagnosis in a significant proportion of patients and up to 10–15% of smear-negative TB [12,13]. Moreover, another study confirmed up 49.7% relative gain and more case detection by means of Xpert as an add-on test, recommending performing Xpert as the first diagnostic test, to avoid extraordinary work load [14].

Culture is regarded as the best available reference standard for active TB disease and was the reference standard for TB in most studies.

In the current study, culture on LJ medium stood as the gold standard for detecting MTB. High sensitivity of Gene Xpert MTB/RIF detected in the current study (98.15%) helps to rule out disease with a high degree of confidence. Its accuracy was 96.55%.

A previous Egyptian study in a cohort of MTB patients recorded that, the sensitivity of Xpert in detection of smear positive, culture positive TB was 100% and in smear negative, culture positive TB was 66.6% while its specificity in both was 100% [15].

Even with Versa TREK considered by El Hossary, as the gold standard test, the author reported the superior sensitivity of Gene Xpert MTB/RIF to detect MTB in smear positive specimens compared to Versa TREK (100% versus 52.3%) in Saudi Arabia [16].

Since 2010, the WHO recommended the Xpert MTB/RIF (Cepheid) assay as a diagnostic tool, being reported in a multi-country study to have sensitivities of 98.2% among smear-positive, culture positive patients and 72.5% among smear negative, culture positive patients on a single direct Xpert MTB/RIF test compared to repeated smears and culture results [17]. Other researchers reported 100% sensitivity and specificity of 86% [13], and 86.9% sensitivity and 99.7% specificity [18].

Xpert MTB/RIF assay did not cross-react with different non-tuberculous mycobacteria (NTM) species tested at high copy numbers, suggesting that the assay would have high specificity not affected by the presence of NTM species or cross-contamination [4]. It is worth noting that Xpert MTB/RIF requires minimal biosafety facilities, allowing sample decontamination, hands-free operation, on-board sample processing. It has a high sensitivity in smear-negative pulmonary TB and ultrasensitive in hemi-nested PCR for smear positive [19].

The superiority of Gene Xpert MTB/RIF assay in retreatment TB cases lies on its ability not only to detect TB bacilli, but in forecasting treatment response to rifampicin as well.

A number of studies have raised concerns about rapid drug susceptibility test (DST) methods, in particular automated MGIT 960, for detection of rifampicin resistance [20].

This study showed a head-to-head comparison between DST results of MGIT 960 as a reference test and Gene Xpert MTB/RIF regarding rifampicin resistance led to equivalent results of both. Rifampicin resistance was detected in 37 (68.5%) indicating that sensitivity and specificity of Gene Xpert MTB/RIF were 100% each. Meanwhile sensitivity and specificity of rifampin resistance in clinical specimens were reported to range from 86% to 100% and 95% to 100%, respectively, with higher sensitivity in smear-positive cases [6].

Moreover, the superiority of Xpert MTB/RIF was noticed in identification of more cases with mutations to the rpoB gene than which appeared to be rifampicin susceptible using MGIT 960 [21]. In addition to the lengthy average time required for completion of the reference test MGIT was 2.5 days [22].

From current operational approach, on the basis of time benefit analysis, Xpert MTB/RIF had the least turnaround time, it took about two hours. On the other hand the less sensitive screening tool, ZN smear consumed more time with false negative results and/or double or triple specimens, meanwhile the gold standard test used, LJ culture took about 4–8 weeks. In agreement with our comment, it was recognized that Gene Xpert testing may have improved detection and time to appropriate treatment for drug-resistant cases, thereby potentially reducing transmission of drug-resistant disease [23].

To sum up, this study highlighted the realistic accuracy of Gene Xpert MTB/RIF assay's sensitivity of 98.15%, specificity of 75% and accuracy of 96.55% in MTB diagnosis. Coupled with absolute sensitivity and specificity 100% each for detection of MTB rifampicin resistance by MGIT. Gene Xpert MTB/RIF should be integrated in diagnostic approach to improve the understanding of the retreatment TB cases. Finally the authors recommend restandardization of reference test with Gene Xpert MTB/RIF to obtain more valid results.

Limitations

Inaccurate history giving of retreatment patients and insufficient documentation in post-discharge file reviews led to underestimated case reporting and relatively small size.

Conflict of interest

No conflict of interest.

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